FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S APPLICATION NO. (IF KNOWN, SEE 37 CFR)
221312US0PCT	10/088525

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/JP00/06873	2 October 2000	12 October 1999

TITLE OF INVENTION
REMEDIES FOR INTRACTABLE WOUND

APPLICANT(S) FOR DO/EO/US
TAKAKURA Shoji et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☒ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☒ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☐ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☐ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

Notice of Priority/PCT/IB/308
PCT/IB/304/Form PTO-1449

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
10/088525	PCT/JP00/06873	221312US0PCT

24. The following fees are submitted:.

BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :

		CALCULATIONS PTO USE ONLY
☐	Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1040.00**	
☒	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$890.00**	
☐	International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$740.00**	
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710.00**	
☐	International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**	
	ENTER APPROPRIATE BASIC FEE AMOUNT =	**$890.00**
	Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$130.00**

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	1 - 20 =	0	x $18.00	**$0.00**	
Independent claims	1 - 3 =	0	x $84.00	**$0.00**	
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**	
	TOTAL OF ABOVE CALCULATIONS =			**$1,020.00**	
☐	Applicant claims small entity status. See 37 CFR 1.27). The fees indicated above are reduced by 1/2.			**$0.00**	
	SUBTOTAL =			**$1,020.00**	
	Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +			**$0.00**	
	TOTAL NATIONAL FEE =			**$1,020.00**	
☐	Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).**			**$0.00**	
	TOTAL FEES ENCLOSED =			**$1,020.00**	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of **$1,020.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **15-0030** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Surinder Sachar
Registration No. 34,423

22850

Surinder Sachar
SIGNATURE

Norman F. Oblon
NAME

24,618
REGISTRATION NUMBER

April 1 2002
DATE

DESCRIPTION

Remedies for intractable wound

TECHNICAL FIELD

This invention relates to a therapeutic drug for refractory injuries, comprising a substance having a human leucocyte elastase inhibitory activity as an effective ingredient.

The inventors of this invention have found that a substance having a human leucocyte elastase inhibitory activity is effective for the treatment of refractory injuries and have completed this invention.

BACKGROUND ART

INDUSTRIAL APPLICABILITY

This invention is a therapeutic drug for refractory injuries, comprising a substance having a human leucocyte elastase inhibitory activity as an effective ingredient.

DISCLOSURE OF THE INVENTION

The substance having a human leucocyte elastase inhibitory activity and being usable as an effective ingredient of a therapeutic drug for refractory injuries may be any substance having a human leucocyte elastase inhibitory activity. Furthermore, the substance having a human leucocyte elastase inhibitory activity and being usable in this invention includes not only substances that directly inhibit leucocyte elastase but also substances that indirectly inhibit leucocyte elastase by suppressing the infiltration of leucocytes or by inhibiting the generation of elastase. In other words, various substances having such an activity are known. Not only the known substances but also new substances can also be used if they have the human leucocyte elastase inhibitory. Among these, particularly

suitable compounds are exemplified below.

(1) WS7622A mono- or disulfate ester and pharmaceutically acceptable salts thereof: among them, the disodium salt of the WS7622A disulfate ester and the dipotassium salt of the WS7622A disulfate ester are known substances having the following physico-chemical properties respectively (Japanese Laid-open Patent Application No. Hei 4-279600).

Disodium salt of WS7622A disulfate ester

Appearance: colorless crystal

Solubility: soluble: water, methanol

insoluble: chloroform, n-hexane

Melting point: 257 to 263°C (dec.)

Specific rotation: $[\alpha]^{23}_{D}$ +37.5° (C=1, methanol)

Molecular formula: $C_{17}H_{61}N_9O_{19}S_2Na_2$

Elemental analysis:

Calcd for $C_{17}H_{61}N_9O_{19}S_2Na_2 \cdot 6H_2O$

C 44.30, H 5.77, N 9.89, S 5.03, Na 3.61 %

Found: C 44.98, H 5.90, N 10.06, S 5.00, Na 3.98 %

Molecular weight: FAB-MS m/z 1188 $(M+Na)^+$

Thin layer chromatography:

Stationary phase	Developing solvent	Rf value
Silica gel (Merck Art 5715)	$CHCl_3$-CH_3OH-H_2O (65 : 25 : 4)	0.11
	n-butanol-acetic acid-water	0.29

Infrared absorption spectrum:

γ^{KBr}_{max}: 3360, 2960, 1735, 1660, 1640, 1530, 1500, 1380, 1250, 1200, 1060, 1030, 940, 890 cm^{-1}

^1H Nuclear magnetic resonance spectrum:

(400 MHz, D_2O) δ

7.50 (1H, s)

7.27 (1H, s)

7.33-7.24 (3H, m)

	6.94	(1H, q, J=7Hz)
	6.85	(2H, br d, J=8Hz)
	5.53	(1H, m)
	5.37	(1H, m)
	4.80	(1H, br s)
	4.63-4.57	(2H, m)
	4.53	(1H, m)
	4.06	(1H, m)
	3.99	(1H, d, J=10Hz)
	3.56	(1H, br d, J=14Hz)
	3.46	(1H, m)
	2.97	(3H, s)
	2.97-2.88	(2H, m)
	2.72	(1H, m)
	2.59	(1H, m)
	2.51-2.38	(2H, m)
	2.09-1.91	(4H, m)
	1.82-1.60	(3H, m)
	1.77	(3H, d, J=7Hz)
	1.50	(3H, d, J=6.5Hz)
	1.40	(1H, m)
	1.11	(6H, d, J=7Hz)
	0.99	(3H, d, J=6.5Hz)
	0.97	(3H, d, J=6.5Hz)

^{13}C Nuclear magnetic resonance spectrum:

(100 MHz, D_2O) δ

183.6	(s)
177.9	(s)
177.7	(s)
174.8	(s)
173.8	(s)
173.3	(s)
172.4	(s)
167.8	(s)
161.5	(s)

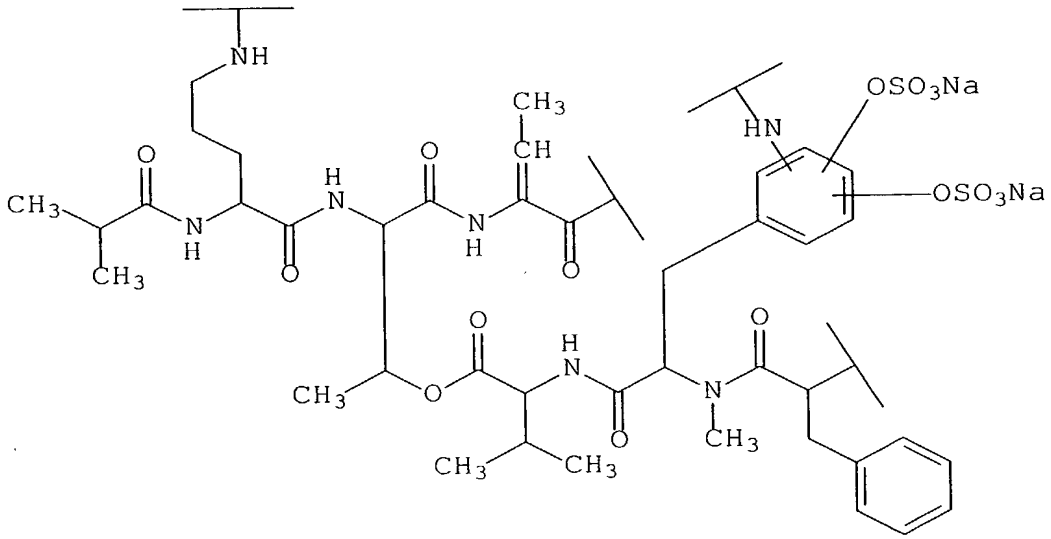
	145.5	(s)
	144.9	(s)
	139.6	(d)
	139.0	(s)
	137.0	(s)
	136.0	(s)
	132.3	(d) x 2
	131.0	(d) x 2
	129.6	(d)
	127.4	(d)
	125.9	(d)
	77.4	(d)
	75.1	(d)
	63.8	(d)
	62.7	(d)
	59.1	(d)
	55.9	(d)
	54.9	(d)
	51.9	(d)
	41.9	(t)
	37.2	(d)
	36.9	(t)
	34.1	(q)
	32.3	(d)
	31.9	(t)
	31.8	(t)
	31.2	(t)
	27.5	(t)
	23.7	(t)
	21.7	(q)
	21.4	(q) x 2
	21.3	(q)
	21.1	(q)
	15.5	(q)

Amino acid analysis

The disodium salt (1 mg) of the WS7622A disulfate ester was hydrolyzed in 6N hydrochloric acid (1 ml) at 110°C for 20 hours, and dried under reduced pressure to obtain a mixture. The mixture was measured by Hitachi 835 Automatic Amino Acid Analyzer. Type H (Wako code: 013-08391) and type B (Wako code: 016-08641) of Wako Pure Chemical Industries, Ltd. were used as standard amino acid samples.

As a result, threonin, valine, phenyl alanine, ornithine, ammonia and several kinds of unknown ninhydrin positive components were detected.

The following formula is proposed as a partial chemical structural formula of the disodium salt of the WS7622A disulfate ester.



Dipotassium salt of the WS7622A disulfate ester

Appearance: colorless amorphous powder

Solubility: soluble: water, methanol

insoluble: chloroform, n-hexane

Melting point: 230 to 237°C (dec.)

Specific rotation: $[\alpha]^{23}_{D}$ +34° (C=1, methanol)

Molecular formula: $C_{17}H_{61}N_9O_{19}S_2K_2$

Elemental analysis:

Calcd for $C_{17}H_{61}N_9O_{19}S_2K_2 \cdot 6H_2O$

C 43.21, H 5.63, N 9.65, S 4.91, K 5.99 %

Found: C 43.96, H 5.44, N 9.97, S 5.09, K 4.49 %

Molecular weight: FAB-MS m/z 1236 $(M+K)^+$

Thin layer chromatography:

Stationary phase	Developing solvent	Rf value
Silica gel (Merck Art 5715)	$CHCl_3$-CH_3OH-H_2O (65 : 25 : 4)	0.13

Infrared absorption spectrum:

γ^{KBr}_{max}: 3360, 2960, 1735, 1660, 1640, 1530, 1500, 1405, 1380, 1250, 1200, 1050, 1030, 910, 890 cm^{-1}

^1H Nuclear magnetic resonance spectrum:

(400 MHz, D_2O) δ

7.52	(1H, s)
7.28	(1H, s)
7.34-7.25	(3H, m)
6.96	(1H, q, J=7Hz)
6.87	(2H, br d, J=8Hz)
5.56	(1H, m)
5.40	(1H, m)
4.84	(1H, br s)
4.70-4.55	(3H, m)
4.10	(1H, m)
4.03	(1H, m)
3.60	(1H, br d, J=14Hz)
3.50	(1H, m)
3.00	(3H, s)
3.00-2.85	(2H, m)
2.76	(1H, m)
2.62	(1H, m)
2.55-2.40	(2H, m)
2.12-1.95	(4H, m)

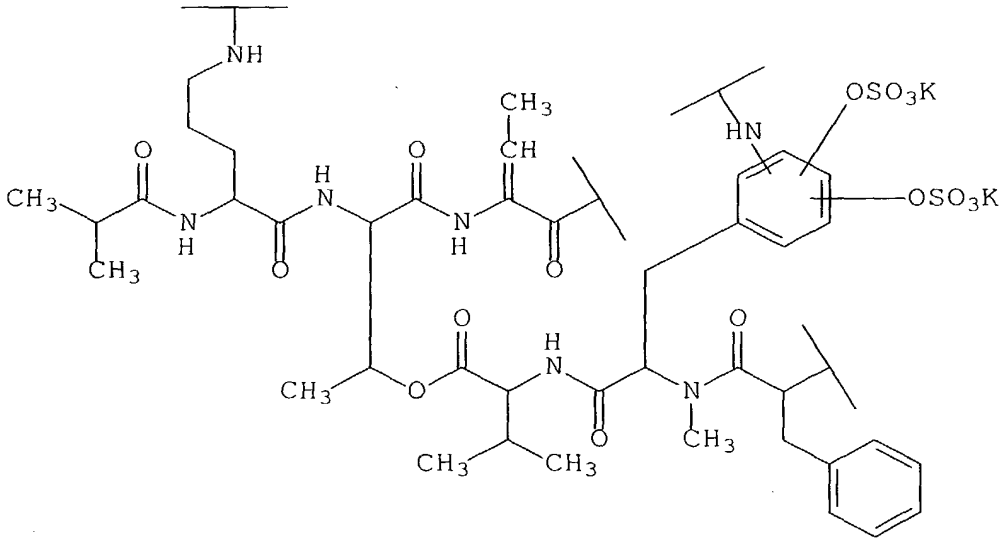
1.90-1.65	(3H,	m)
1.79	(3H,	d, J=7Hz)
1.53	(3H,	d, J=6.5Hz)
1.45	(1H,	m)
1.14	(6H,	d, J=7Hz)
1.02	(3H,	d, J=6.5Hz)
1.00	(3H,	d, J=6.5Hz)

Amino acid analysis

The dipotassium salt (1 mg) of the WS7622A disulfate ester was hydrolyzed in 6N hydrochloric acid (1 ml) at 110°C for 20 hours, and dried under reduced pressure to obtain a mixture. The mixture was measured by Hitachi 835 Automatic Amino Acid Analyzer. Type H (Wako code: 013-08391) and type B (Wako code: 016-08641) of Wako Pure Chemical Industries, Ltd. were used as standard amino acid samples.

As a result, threonin, valine, phenyl alanine, ornithine, ammonia and several kinds of unknown ninhydrin positive components were detected.

The following formula is proposed as a partial chemical structural formula of the dipotassium salt of the WS7622A disulfate ester.



Pharmaceutically acceptable salts of the WS7622A mono- or disulfate ester may include a mono- or disalt with an inorganic or organic base, for example, an alkali metal salt (e.g., sodium salt, potassium salt, etc.), an alkaline earth metal salt (e.g., calcium salt, etc.), an ammonium salt, an ethanolamine salt, a triethylamine salt, a dicyclohexylamine salt, a pyridine salt, etc.

The WS7622A substance, a starting substance for the synthesis of the above-mentioned WS7622A mono- or disulfate ester, also has the human leucocyte elastase inhibitory activity and can be used as a therapeutic drug for refractory injuries. The substance is known as a substance having the following physico-chemical properties (Japanese Laid-open Patent Application No. Hei 3-218387 and Japanese Laid-open Patent Application No. Hei 4-279600).

Physico-chemical properties of the WS7622A substance
Appearance: colorless prism crystal
Property of substance: acidic
Color reaction:
Positive: cerium sulfate reaction, iodine vapor reaction, ferric chloride reaction
Negative: ninhydrin reaction, Molisch reaction, Dragendorff reaction
Solubility: soluble: methanol, ethanol, n-butanol
slightly soluble: chloroform, acetone, ethyl acetate
insoluble: water, n-hexane
Thin layer chromatography (TLC):
Chloroform-methanol (5 : 1, v/v)
Rf value 0.51
Acetone-methanol (10 : 1)
Rf value 0.62
(Kiesel gel $60F_{251}$ silica gel plate, Merck)

Melting point: 250 to 252°C (dec.)

Specific rotation: $[\alpha]^{23}_{D}$ +36° (C=1, methanol)

UV spectrum: λ^{MeOH}_{max} 287 nm (ξ = 3600)

$\lambda^{MeOH-HCl}_{max}$ 287 nm

$\lambda^{MeOH-NaOH}_{max}$ 298 nm

Molecular formula: $C_{17}H_{63}N_9O_{13}$

Elemental analysis:

Calcd for $C_{17}H_{63}N_9O_{13}\cdot 2H_2O$

C 56.56, H 6.77, N 12.63 %

Found: C 56.65, H 6.62, N 12.27 %

Molecular weight: FAB-MS m/z 984 $(M+Na)^+$

Infrared absorption spectrum:

γ^{KBr}_{max}: 3400, 3300, 3060, 2980, 2940, 1735, 1710, 1690, 1670, 1660, 1640, 1540, 1520, 1470, 1380, 1330, 1300, 1260, 1220, 1200, 1160, 1130, 1090, 1000, 980, 940, 920 cm^{-1}

^1H Nuclear magnetic resonance spectrum:

(400 MHz, CD_3OD) δ

7.22-7.09	(3H,	m)
6.88-6.77	(3H,	m)
6.74	(1H,	s)
6.46	(1H,	s)
5.46	(1H,	m)
5.18	(1H,	s)
4.85	(1H,	s)
4.77	(1H,	m)
4.65	(1H,	m)
4.50	(1H,	m)
3.96	(1H,	m)
3.91	(1H,	d, J=9Hz)
3.60-3.47	(2H,	m)
3.03	(1H,	m)
2.90	(3H,	s)
2.86	(1H,	m)

2.59-2.49	(2H, m)	
2.39	(1H, m)	
2.29-2.16	(2H, m)	
2.00	(1H, m)	
1.84	(1H, m)	
1.74	(3H, d, J=6Hz)	
1.72-1.53	(4H, m)	
1.44	(3H, d, J=6Hz)	
1.12	(1H, m)	
1.10	(6H, d, J=6Hz)	
0.99	(3H, d, J=6Hz)	
0.94	(3H, d, J=6Hz)	

^{13}C Nuclear magnetic resonance spectrum:

(100 MHz, CD_3OD)	δ	
179.7	(s)	
176.3	(s)	
174.7	(s)	
173.3	(s)	
172.4	(s)	
171.4	(s)	
170.3	(s)	
165.8	(s)	
160.2	(s)	
145.7	(s)	
145.6	(s)	
137.5	(s)	
134.0	(d)	
131.4	(s)	
130.6	(d)	x 2
129.8	(s)	
129.1	(d)	x 2
129.1	(s)	
127.6	(d)	
119.1	(d)	
118.0	(d)	

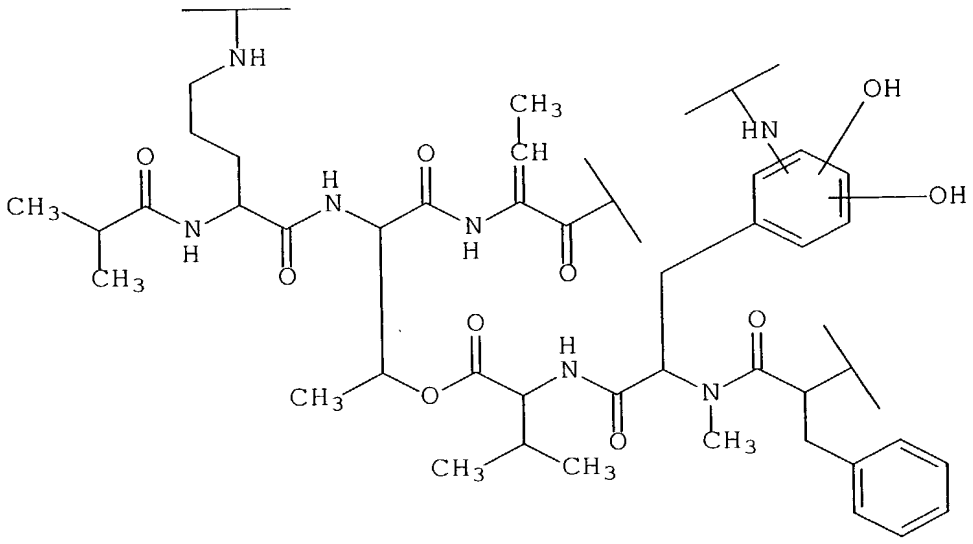
76.0	(d)	
73.4	(d)	
63.1	(d)	
61.4	(d)	
57.1	(d)	
53.6	(d)	
52.7	(d)	
50.5	(d)	
39.9	(t)	
36.1	(t)	
35.8	(d)	
31.8	(q)	
31.0	(t)	
30.8	(d)	
29.9	(t)	
29.7	(t)	
25.2	(t)	
22.3	(t)	
20.2	(q)	
20.0	(q)	x 2
19.7	(q)	
19.5	(q)	
13.3	(q)	

Amino acid analysis

WS7622A(1 mg) was hydrolyzed in 6N hydrochloric acid (1 ml) at 110°C for 20 hours, and dried under reduced pressure to obtain a mixture. The mixture was measured by Hitachi 835 Automatic Amino Acid Analyzer. Type H (Wako code: 013-08391) and type B (Wako code: 016-08641) of Wako Pure Chemical Industries, Ltd. were used as standard amino acid samples.

As a result, threonin, valine, phenyl alanine, ornithine, ammonia and several kinds of unknown ninhydrin positive components were detected.

The following formula is proposed as a partial chemical structural formula of the WS7622A.

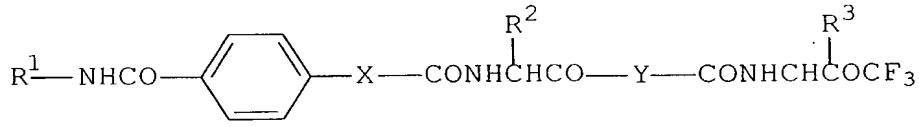


Salts of the WS7622A substance may include a salt with an inorganic or organic base, for example, an alkali metal salt (e.g., sodium salt, potassium salt, etc.), an alkaline earth metal salt (e.g., calcium salt, etc.), an ammonium salt, an ethanolamine salt, a triethylamine salt, a dicyclohexylamine salt, etc.

Similarly, WS7622B, WS7622C and WS7622D substances and their derivatives (Japanese Laid-open Patent Application No. Hei 3-218387), having the human leucocyte elastase inhibitory activity, can also be used as therapeutic drugs for refractory injuries.

The above-mentioned WS7622A substance (similarly, WS7622B, WS7622C and WS7622D substances) can be produced by culturing the streptomyces resistomycificus No. 7622 strain, for example. The fungal strain was deposited with National Institute of Bioscience and Human-Technology, an international depository authority on the Budapest Treaty, under the deposit number FERM BP-2306.

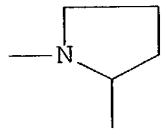
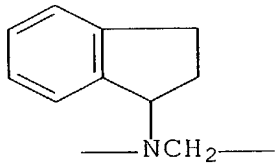
(2) Trifluoromethylketone derivative represented by the following formula:



in which R^1 is lower alkyl having one or two substituents selected from a group consisting of carboxy, esterified carboxy and di-lower alkylcarbamoyl; phenyl(lower)alkyl which may have halogen, amino or nitro at the phenyl moiety and may have carboxy or esterified carboxy at the alkyl moiety; halophenyl; morpholino; or morpholino(lower)alkyl,

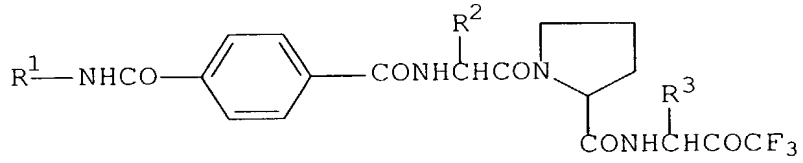
R^2 and R^3 are each lower alkyl,

X is - or -NH-,

Y is  or  ,

and a pharmaceutically acceptable salt thereof.

(3) Trifluoromethylketone derivative represented by the following formula:



in which R^1 to R^3 are the same as those of the above-mentioned compound (2),
and a pharmaceutically acceptable salt thereof.

(4) 3(RS)-[[4-(carboxymethylaminocarbonyl)phenylcarbonyl]-L-valyl-L-prolyl]amino-1,1,1-trifluoro-4-methyl-2-oxopentane or

a sodium salt thereof

The compounds described at the above items (2) to (4) are known compounds described in Japanese Laid-open Patent Application No. Hei 4-297446. In addition, pharmaceutically acceptable salts of the compounds described at the items (2) to (4) may include a salt with an inorganic or organic base, for example, an alkali metal salt (e.g., sodium salt, potassium salt, etc.), an alkaline earth metal salt (e.g., calcium salt, etc.), an ammonium salt, an ethanolamine salt, a triethylamine salt, a dicyclohexylamine salt, etc., and an organic or inorganic acid addition salt, for example, methanesulfonate, hydrochloride, sulfate, nitrate, phosphate, etc.

Suitable examples of the above-mentioned definitions are explained in detail as follows.

The term "lower" is intended to means 1 to 6 carbon atoms, unless otherwise indicated.

Suitable examples of "halogen" may include fluorine, chlorine, bromine and iodine.

Suitable "lower alkyl" may include a straight or branched alkane residue having 1 to 6 carbon atoms, such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, t-butyl, pentyl, neo-pentyl, hexyl and the like, preferably those having 1 to 4 carbon atoms.

Suitable "esterified carboxy" may be alkyl ester, that is, alkoxycarbonyl, for example, lower alkoxycarbonyl (e.g. methoxycarbonyl, ethoxycarbonyl, propoxycarbonyl, butoxycarbonyl, tert-butoxycarbonyl, etc.) and phenyl(lower)alkyl ester, that is, phenyl(lower)alkoxy carbonyl, for example, benziloxycarbonyl, and benzoyl(lower)alkyl ester, that is, benzoyl(lower)alkoxy carbonyl, for example, benzoylmethoxycarbonyl, etc.

Suitable "lower alkylene" may include methylene,

ethylene, propylene, isopropylene, etc.

Suitable "di-lower alkylcarbamoyl" may include N,N-dimethylcarbamoyl, N,N-diethylcarbamoyl, etc.

(5) FR901451 substance having the following physico-chemical properties and a pharmaceutically acceptable salt thereof

Appearance: white powder

Color reaction:

Positive: cerium sulfate, iodine vapor, Ehrlich, ninhydrin

Negative: Molisch

Solubility: soluble: water, methanol, dimethyl sulfoxide
hardly soluble: acetone
insoluble: ethyl acetate

Melting point: 243 to 245°C (dec.)

Specific rotation: $[\alpha]^{23}_{D}$ -15° (C=0.65, H_2O)

UV absorption spectrum: λ^{MeOH}_{max} nm (ξ) 275 = (4300)
281 (4500), 290 (3900)

Molecular formula: $C_{60}H_{79}N_{13}O_{18}$

Elemental analysis:

Calcd for $C_{60}H_{79}N_{13}O_{18}\cdot 10H_2O$
C 49.68, H 6.88, N 12.55 %

Found: C 49.95, H 6.28, N 12.42 %

Molecular weight: FAB-MS m/z 1270 $(M+H)^+$

Thin layer chromatography:

Stationary phase	Developing solvent	Rf value
Silica gel (Merck)	$CHCl_3$: MeOH: NH_4OH (15 : 11 : 5)	0.60
RP-18 (Merck)	70% hydrous methanol	0.32

FT Infrared absorption spectrum:

γ^{KBr}_{max}: 3390, 3070, 2970, 2880, 1740, 1660, 1530, 1450, 1410, 1380, 1350, 1250, 1190, 1110, 1080, 1010, 750, 700, 670, 660, 620, 600 cm^{-1}

^{1}H Nuclear magnetic resonance spectrum:

(400 MHz, D_2O) δ

7.70	(1H, d, J=7Hz)
7.52	(1H, d, J=7.5Hz)
7.44-7.23	(7H, m)
7.22	(1H, s)
5.59	(1H, q, J=7Hz)
4.94	(1H, t, J=4.5Hz)
4.85-4.74	(3H, m)
4.58	(1H, dd, J=6Hz, 10Hz)
4.45-4.35	(3H, m)
4.30	(1H, dd, J=4Hz, 7Hz)
4.07	(1H, m)
3.99	(1H, dd, J=10Hz, 4.5Hz)
3.66-3.50	(3H, m)
3.44-3.25	(4H, m)
3.16-2.93	(4H, m)
2.87	(1H, d, J=18Hz)
2.80-2.68	(2H, m)
2.56-2.48	(2H, m)
2.08	(1H, dd, J=16Hz, 4Hz)
1.87-1.53	(9H, m)
1.43	(3H, d, J=7Hz)
1.30	(3H, d, J=6.5Hz)
1.45-1.17	(4H, m)
0.95	(3H, d, J=6Hz)
0.84	(3H, d, J=6Hz)

^{13}C Nuclear magnetic resonance spectrum:

(100 MHz, D_2O) δ

177.2 (s)	130.0 (d) x 2	56.0 (d)	31.4 (t)
176.5 (s)	129.8 (d) x 2	54.1 (d)	28.8 (t)
174.6 (s)	128.5 (d)	53.8 (d)	26.6 (t)
174.2 (s)	127.8 (d)	53.2 (d)	25.1 (d)
174.0 (s)	125.5 (d)	53.1 (d)	23.2 (q)
173.2 (s)	123.2 (d)	52.9 (d)	23.2 (t)

173.0 (s)	120.9 (d)	52.8 (d)	23.1 (t)
172.8 (s)	118.7 (d)	49.5 (d)	20.8 (q)
172.6 (s)	113.1 (d)	48.6 (t)	19.4 (q)
172.5 (s)	108.8 (s)	40.1 (t)	18.3 (q)
172.1 (s)	73.3 (d)	39.6 (t)	
171.7 (s)	69.7 (d)	39.4 (t)	
171.4 (s)	64.3 (d)	38.9 (t)	
170.3 (s)	62.1 (d)	35.3 (t)	
137.2 (s)	60.9 (d)	34.8 (t)	
136.0 (s)	57.1 (d)	31.7 (t)	

The above-mentioned FR90145 substance is known as a substance produced from the FR90145 substance producing fungus of the flexibacter genus (for example, International Publication No. WO93/02203). In addition, the flexibacter sp No. 758 strain of the producing fungus was deposited with National Institute of Bioscience and Human-Technology, an international depository authority on the Budapest Treaty, under the deposit number FERM BP-3420.

Furthermore, pharmaceutically acceptable salts of the above-mentioned FR90145 substance may be the same as the pharmaceutically acceptable salts of the compounds described at the above-mentioned items (2) to (4).

In addition to those described above, examples of substances having the elastase inhibitory activity may include α1-antitrypsin, SLP1 (Secretory Leukocyte Protease Inhibitor) (American Review of Respiratory Disease Vol. 147, 1993, P442-446), urinastatin, colchicine, erythromycin, clarithromycin, IC1200, 800, ONO-5046 (American Journal of Respiratory and Critical Care Medicine Vol. 153, P391-397), antielastase antibody, etc.

Examples of refractory injuries in accordance with this invention may include ulcers at skin (e.g. decubitus (bedsore), foot ulcers associated with diabetes, etc.),

ulcers at feet, stomach, cornea, etc. and the like. The therapeutic drug for refractory injuries in accordance with this invention is particularly suited for the treatment of refractory skin ulcers, such as foot ulcers associated with diabetes, among the above-mentioned ulcers.

The therapeutic drug for refractory injuries in accordance with this invention is usually used as external preparations (e.g. lotions, ointments, plasters, liniments, aerosols, suspensions, emulsions, etc.) in the case of refractory skin ulcers, for example. In addition, the therapeutic drug can be used in the forms of conventional pharmaceutical preparations, such as powders, fine granules, granules, tablets, dragees, injection solutions, insufflations, microcapsules, capsules, suppositories, solutions, syrups, etc.

If necessary, there may be included in the above preparations diluents, disintegrating agents (e.g. sucrose, starch, crystalline cellulose, L-hydroxypropylcellulose, synthetic aluminum silicate, etc.), binders (e.g. cellulose, methylcellulose, hydroxypropylcellulose, hydroxypropylmethylcellulose, polypropylpyrrolidone, polyvinylpyrrolidone, gelatin, gum Arabic, polyethylene glycol, etc.), coloring agents, sweeteners, lubricants (e.g. magnesium stearate, etc.) and the like.

While the dosage of the therapeutic drug for refractory injuries in accordance with this invention varies depending on the condition and the like of each patient to be treated, in the case of external administration, a dose of about 0.001-10% of the substance having a human leucocyte elastase inhibitory activity or a pharmaceutically acceptable salt thereof should be used generally.

Next, the effects of this invention are described by using a test example.

Test example (diabetic rat foot ulcer curing action)

Purpose:

The action of the compound (applied) in accordance with this invention on a foot ulcer induced by acetic acid was examined by using normal and diabetic rats.

Compound used for the test:

Sodium salt of 3(RS)-[[4-(carboxymethylaminocarbonyl) phenylcarbonyl]-L-valyl-L-prolyl]amino-1,1,1-trifluoro-4-methyl-2-oxopentane (FR136706)

Method:

Diabetes was induced in each of a seven-week-old male SD rats by intravenously administrating 60 mg/kg streptozotocin (STZ) to its tail. Fourteen days after the administration of STZ, 20 µl glacial acetic acid was administered into the skin of the left foot instep of each of the diabetic rats and control rats of the same age while anesthetized using ether, thereby causing necrosis at the portion. In the case when the necrotic cuticle of the skin remained two days after the necrosis, the cuticle was removed surgically. Then, the administration of FR136706 (0.2% solution in PEG (polyethylene glycol) 400) was started (50 µl to the affected portion). PEG400 was administered to the control group in a similar way.

In a period between two days and 25 days after the administration of acetic acid, swelling scores (0: no swelling, 1: slight swelling, 2: intermediate swelling, 3: significant swelling) was checked visually, and the major axis length and the minor axis length of each ulcer was measured with vernier calipers. The area of each ulcer was calculated from the major axis length and the minor axis length thereof.

Result:

The swelling scores of the normal rats were highest on the measurement start day. Then, the rats were recovered and their scores became zero 22 days after the administration of the acetic acid. On the other hand, in the case of the diabetic rats, the peaks of the swelling scores were found seven days after the administration of the acetic acid. Although the rats were recovered gradually after that, the progress of the recovery was slower than that of the normal rats. FR136706 did not act on the normal rats, but promoted the recovery of the diabetic rats.

The swelling areas of the diabetic rats were larger than those of the normal rats, and the contraction of the areas of the diabetic rats was slower than that of the normal rats. FR136706 did not act on the normal rats, but it was recognized that FR136706 tended to promote the contraction of the ulcer areas of the diabetic rats.

Action on foot ulcer models

Animal	Specimen	Dosage (%)	Score: Swelling score after administration of acetic acid						
			After 2 days	After 8 days	After 11 days	After 15 days	After 18 days	After 22 days	After 25 days
Normal rat	PEG 400		2.5 ±0.2 (6)	2.3 ±0.2 (6)	1.8 ±0.2 (6)	1.0 ±0.0 (6)	0.3 ±0.2 (6)	0.0 ±0.0 (6)	0.0 ±0.0 (6)
	FRI 136706	0.2	2.5 ±0.2 (6)	2.0 ±0.2 (6)	1.5 ±0.2 (6)	1.0 ±0.0 (6)	0.5 ±0.2 (6)	0.0 ±0.0 (6)	0.0 ±0.0 (6)
Diabetic rat	PEG 400		2.2 ±0.2 (6)	2.8 ±0.2 (6)	* 2.7 ±0.2 (6)	** 2.2 ±0.3 (6)	** 2.0 ±0.3 (6)	** 1.7 ±0.3 (6)	** 1.5 ±0.2 (6)
	FRI 136706	0.2	2.2 ±0.2 (6)	2.8 ±0.2 (6)	2.5 ±0.2 (6)	1.5 ±0.2 (6)	1.5 ±0.2 (6)	1.2 ±0.2 (6)	& 0.7 ±0.2 (6)

Average ± standard error (n)

&, &&: significant at 5% and 1% respectively (Wilcoxon Rank Sum Test)

[Score]

[Scores of PEG400 group of diabetic rats and FRI136706 0.2% group of diabetic rats on each measurement day]

*, **: significant at 5% and 1% respectively (Wilcoxon Rank Sum Test)

[Score]

[Scores of PEG400 group of normal rats and PEG400 group of diabetic rats on each measurement day]

Action on foot ulcer models

Animal	Specimen	Dosage (%)	Ulcer area (mm^2) after administration of acetic acid						
			After 2 days	After 8 days	After 11 days	After 15 days	After 18 days	After 22 days	After 25 days
Normal rat	PEG 400		58.88 ±4.31 (6)	70.29 ±6.13 (6)	52.61 ±6.36 (6)	24.99 ±2.82 (6)	1.51 ±0.78 (6)	0.00 ±0.00 (6)	0.00 ±0.00 (6)
	FRI 136706	0.2	58.37 ±6.08 (6)	71.42 ±8.43 (6)	53.21 ±5.11 (6)	18.32 ±4.55 (6)	0.69 ±0.36 (6)	0.00 ±0.00 (6)	0.00 ±0.00 (6)
Diabetic rat	PEG 400		69.28 ±5.33 (6)	95.58* ±8.62 (6)	86.03** ±7.71 (6)	51.63** ±6.12 (6)	23.38** ±1.42 (6)	15.94 ±3.90 (6)	11.05 ±1.68 (6)
	FRI 136706	0.2	69.17 ±5.64 (6)	91.77 ±6.16 (6)	72.38 ±10.37 (6)	41.00 ±10.80 (6)	16.10 ±6.43 (6)	12.08 ±3.73 (6)	6.99 ±1.71 (6)

Average ± standard error (n)

*, **: significant at 5% and 1% respectively (Student-t or Aspin-Welch)

[Ulcer area]

[PEG400 group of normal rats and PEG400 group of diabetic rats on each measurement day]

CLAIMS

1. A therapeutic drug for refractory injuries, comprising a substance having a human leucocyte elastase inhibitory activity as an effective ingredient.

ABSTRACT

This invention provides a therapeutic drug for refractory injuries, comprising a substance having a human leucocyte elastase inhibitory activity as an effective ingredient.

Declaration, Power of Attorney and Petition

WE (I) the undersigned inventor(s), hereby declare(s) that:

My residence, post office address and citizenship are as stated below next to my name,

We (I) believe that we are (I am) the original, first and joint (sole) inventor(s) of the subject matter which is claimed and for which a patent is sought on the invention entitled

REMEDIES FOR INTRACTABLE WOUND

the specification of which

☐ is attached hereto.

☐ was filed on _____ as

Application Serial No. _____

and amended on _____.

☒ was filed as PCT international application

Number PCT/JP00/06873

on October 2, 2000,

and was amended under PCT Article 19

on _____ (if applicable).

We (I) hereby state that we (I) have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

We (I) acknowledge the duty to disclose information known to be material to the patentability of this application as defined in Section 1.56 of Title 37 Code of Federal Regulations.

We (I) hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed. Prior Foreign Application(s)

Application No.	Country	Day/Month/Year	Priority Claimed
11/289247	JAPAN	12/10/99	☒ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No
			☐ Yes ☐ No

We (I) hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

(Application Number) (Filing Date)

(Application Number) (Filing Date)

We (I) hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or under § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application Serial No.	**Filing Date**	**Status (pending, patented, abandoned)**
PCT/JP00/06873	October 2, 2000	

And we (I) hereby appoint the following registered practitioner(s):

22850

as our (my) attorneys, with full powers of substitution and revocation, to prosecute this application and to transact all business in the Patent Office connected therewith; and we (I) hereby request that all correspondence regarding this application be sent to

22850

We (I) declare that all statements made herein of our (my) own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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